



ELSEVIER

MARINE
ENVIRONMENTAL
RESEARCH

Marine Environmental Research 50 (2000) 135–139

www.elsevier.com/locate/marenvrev

Exposure to environmentally relevant concentrations of different nonylphenol formulations in Japanese medaka

C.M. Foran ^{*}, E.R. Bennett ¹, W.H. Benson ²

Environmental and Community Health Research, The University of Mississippi, University, MS 38677, USA

Accepted 5 May 2000

Abstract

The time course of exposure to *p*-nonylphenol (NP) from two different sources was compared to equivalent exposures of 17- β -estradiol (E2) and a solvent control (ethanol; EtOH). Japanese medaka were exposed for 4 days to a nominal concentration of 20 μ g/l of either NP-I (Schenectady International, Inc.), NP-II (Aldrich), or E2, and were then placed in untreated water for 5 days. Tissue samples were taken at two time points during the 4-day exposure and two time points during the 5 days following exposure. Liver homogenates were analyzed using a western blot to detect vitellogenin (VTG) and quantified by measuring the optical density for each lane. Preliminary results indicate that E2 significantly increased VTG staining above the level observed in EtOH-treated controls for both males and females. A two-way analysis of variance (ANOVA) indicates that NP from both sources, as well as E2, significantly increased VTG staining in males (ANOVA, $n=48$, $P<0.001$; Tukey pairwise tests, all $P<0.008$). A significant increase in VTG was observed in E2-treated males and females the first day following transfer into toxicant-free water (two-way ANOVAs, both $n=48$, $P<0.003$; Tukey pairwise tests, all $P<0.019$). If confirmed, this extended response observed for low-level exposures may represent a significant factor for sampling scenarios following pulsed exposure. © 2000 Elsevier Science Ltd. All rights reserved.

^{*} Corresponding author. Tel.: +1-601-232-5958; fax: +1-601-232-1285.

E-mail address: cmforan@olemiss.edu (C.M. Foran).

¹ Present address: Environmental Protection, Canadore College, North Bay, Ontario, Canada P1B 8K9.

² Present address: Gulf Ecology Division, US EPA, 1 Sabine Island Drive, Gulf Breeze, FL 32561-5299, USA.

Nonylphenol (NP) and its ethoxylates are widely used nonionic surfactants, and exposure of aquatic organisms to these chemicals has raised some concern over their potential to act as endocrine disruptors (reviewed in Servos, 1999). NP and other alkylphenol polyethoxylates (APEs) have been detected in rivers and sediments in the UK at concentrations as high as 76 $\mu\text{g/l}$ (Blackburn, Kirkby & Waldock, 1999) and in the USA at concentrations of 0.64 $\mu\text{g/l}$ with slightly higher concentrations found in sediments (Naylor et al., 1992). A number of other reports reviewed by Bennie (1999) have discovered detectable concentrations of APEs, including NP, at ranges which include 20 $\mu\text{g/l}$ — the reported lowest observable effect level (LOEL) for rainbow trout (Jobling, Sheahan, Osborne, Mattiessen & Sumpter, 1996).

Technical NP is synthesized as a mixture of isomers and may include several by-products of the synthesis process including *ortho*-NP, dinonylphenol, phenol, and 1-nonene. Potentially the composition of isomers and by-products may vary in each NP formulation. For this study we obtained NP from two sources, Schenectady International, Inc. (NP-I) and Alderich (NP-II). Hence, the two aims of this study were to identify the expression of the egg yolk protein vitellogenin (VTG) in response to low-level, short-duration NP exposure, and to compare that response for NP from two different manufacturers.

Adult Japanese medaka were exposed for 4 days to a nominal concentration of 20 $\mu\text{g/l}$ NP-I, NP-II, estradiol (E2), or solvent-only (EtOH) followed by transfer into untreated water and recovery for 5 days. Pairs of adult animals were housed in 800 ml of balanced salt solution (BSS) at 26°C under a constant light cycle (16 h light:8 h dark). Each pair was fed brine shrimp twice daily. Each morning after feeding, 80% of the BSS was renewed and the pair was dosed with 50 μl of solvent containing the appropriate chemical (no solvent was added after the exposure period). Three pair of males ($n=3$) and three pairs of females ($n=3$) were collected on the first and fourth day of the exposure and on days 1 and 5 of the recovery. The animals were sacrificed by anesthizing them in methanesulfonate salt (MS222; Sigma Chemical), followed by decapitation. The livers from each pair of animals were removed and pooled for VTG analysis. For each sample, 20 μg of protein was analyzed by western blot. VTG was detected using an antibody obtained from N. Denslow (University of Florida). The integrated optical density of the lane corresponding to each sample was measured using Scion Image as a relative indication of VTG staining. Trends in VTG induction were examined by a two-way analysis of variance (ANOVA) (factors: four chemical treatments, four time endpoints).

The results for males and females indicated no significant differences in response to NP from two different sources. In addition, although these formulations were used as different treatments, preliminary results from gas chromatograph-mass spectrometry indicated that there were no differences between the two NPs analyzed. In an analysis performed by the Huntsman Corporation, the concentrations of different NP isomers and by-products of synthesis were similar for the two NP samples.

The VTG results indicate that low-level exposure to NP and E2 will induce VTG induction in males relative to solvent-treated controls (ANOVA, $P<0.001$; Tukey pairwise tests, all $P<0.008$; Fig. 1). Among females, neither source of NP resulted in

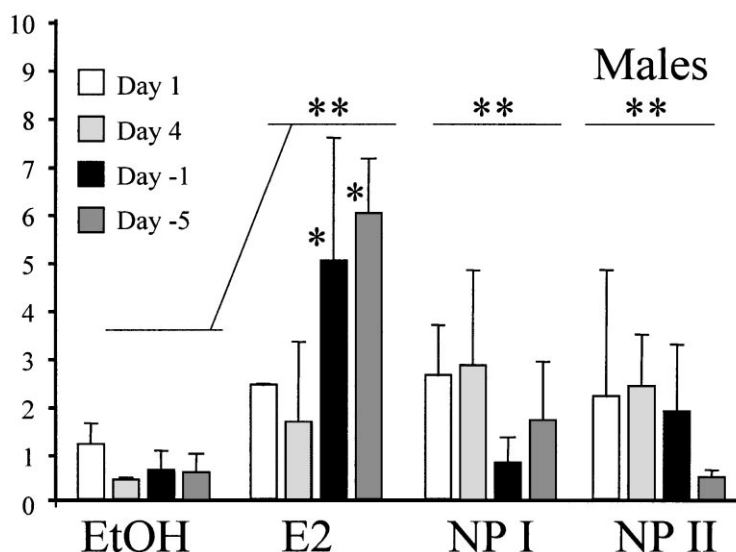


Fig. 1. The optical density of vitellogenin (VTG) staining from liver samples of pairs of male medaka. Each bar shows the mean and standard deviation for each treatment group (clustered along the x axis) and time point ($n=3$ samples at each time point). Two-way ANOVA analysis of VTG staining suggests that *p*-nonylphenol (NP) from both sources as well as 17- β -estradiol (E2) resulted in an increase in VTG staining (**). In addition, VTG staining was found to increase 5 days following the transfer to untreated water (–5; *) relative to the level found in samples collected during exposure (1, 4).

a significantly increased VTG staining above the level observed in ethanol-treated controls; the only significant difference observed between treatment groups was the higher optical density reading recorded in bands from E2-treated females compared to EtOH-treated controls (ANOVA, $P<0.001$; Tukey pairwise test, $P<0.001$; Fig. 2). Interestingly, among E2-treated groups but not NP-treated groups, a significant increase in VTG was observed following transfer to untreated water (ANOVAs, both interaction $P<0.003$; Tukey pairwise tests, all $P<0.02$). For females, tissue samples taken after 1 day in E2-free water exhibited a higher level of VTG staining than those taken after a 24-h exposure (Tukey pairwise test, $P=0.019$; Fig. 2). In males, VTG staining was higher following 5 days in untreated water than in samples taken during the exposure (Tukey pairwise tests, all $P<0.005$; Fig. 1). As well, tissue sample from males collected the day after the exposure ended contained significantly more VTG than samples taken during the exposure 24 h earlier (Tukey pairwise test, $P=0.008$).

These data suggest that low-level exposure resulting in the production of VTG may have subtly different biological effects on males and females. In this preliminary data, males were more sensitive to low concentrations of NP than females. Additionally, in samples collected following exposure to E2, the response of females was more similar to control levels following 5 days in untreated water while males continued to show heightened VTG response. It is likely that females are more

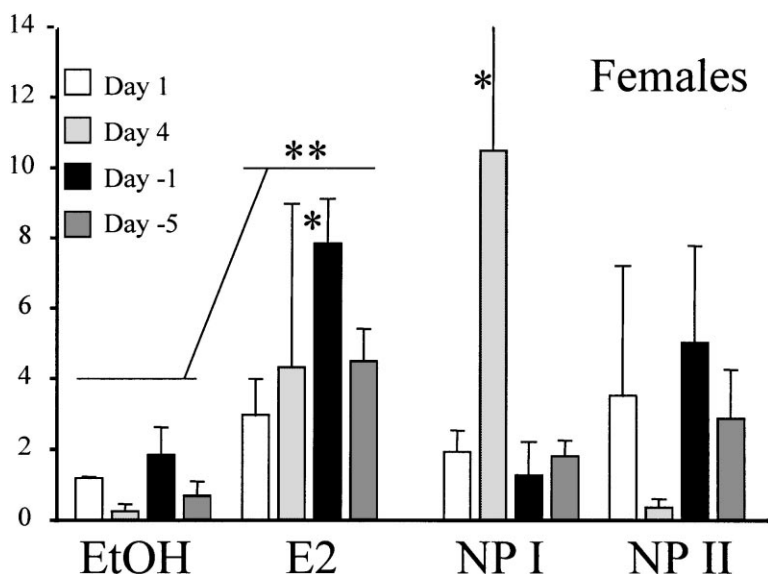


Fig. 2. The optical density of vitellogenin (VTG) staining from liver samples of pairs of female medaka. Each bar shows the mean and standard deviation for each treatment group (clustered along the *x* axis) and time point ($n=3$ samples at each time point). Two-way ANOVA analysis of VTG staining suggests that only 17- β -estradiol (E2) resulted in an increase in VTG staining (**). VTG staining was found to be higher the day following exposure (-1) relative to the concentration detected after the first day of exposure (1; *).

metabolically equipped to cope with increased circulation and deposition of VTG and/or higher levels of estrogenic substances. If confirmed, the faster 'recovery' of females observed for low-level exposures may represent a significant consequence for the timing of sampling in realistic exposure scenarios. For example, these results suggest that for sampling to detect the estrogenic response of animals in the field, female tissue should be collected immediately following pulsatile exposure while male tissue should be sampled over a longer time course. Currently, experiments are being performed to confirm the pattern of VTG concentration in response to low-level, environmentally realistic exposure as well as compare the time course of the response to higher concentrations of NP known to induce VTG.

Acknowledgments

Special thanks to Carter Naylor (Huntsman Corp., Austin, TX) for the analysis of nonylphenol for purity, and to the reviewer for helpful suggestions. Although the research described in this manuscript has been funded, in part, by the US Environmental Protection Agency through grant R 827098-01-0, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

References

- Bennie, D. T. (1999). Review of the environmental occurrence of alkylphenols and alkylphenol ethoxylates. *Water Quality Research Journal of Canada*, 34, 79–122.
- Blackburn, M. A., Kirby, S. J., & Waldock, M. J. (1999). Concentrations of alkylphenol polyethoxylates entering UK estuaries. *Marine Pollution Bulletin*, 38, 109–118.
- Jobling, S., Sheahan, D., Osborne, J. A., Mattiessen, P., & Sumpter, J. P. (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynch mykiss*) exposed to estrogenic alkophenolic chemicals. *Environmental Toxicology and Chemistry*, 15, 194–202.
- Naylor, C. G., Mieure, J. P., Adams, W. J., Weeks, J. A., Castaldi, F. J., Ogle, L. D., & Romano, R. R. (1992). Alkylphenol ethoxylates in the environment. *Journal of American Oil Chemical Society*, 69, 695–703.
- Servos, M. R. (1999). Review of the aquatic toxicity, estrogenic responses, and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. *Water Quality Research Journal of Canada*, 34, 123–177.